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REGENERATION IN THYONE BRIAREUS (LESUEUR) FOLLOWING INDUCED AUTOTOMY

A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY
1934

By
FRANK R. KILLE

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FRANK R. KILLE

(*From Hull Laboratory, University of Chicago, and the Marine Biological Laboratory, Woods Hole, Mass.*)

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Introduction

It has long been known that many holothurians have the ability to cast out a large portion of their viscera and to regenerate all the lost organs (Dalyell, 1840, 1851; Semper, 1868; Noll, 1881; Scott, 1914; Crozier, 1915; Bertolini, 1930, 1932). Although the fact of such regeneration is well known, there have been but few attempts to analyze the manner in which autotomized parts are reconstituted (Scott, 1914; Bertolini, 1930, 1932), and there is even less data on the mode of regeneration following experimental operations (Monticelli, 1896; Torelle, 1909). During the summers of 1931 and 1932 I had an opportunity at Woods Hole[1] to make such investigations upon *Thyone briareus,* a holothurian common to that region.

Our knowledge of the way in which the lost parts are reconstituted in *Thyone briareus* is confined to the accounts of Torelle (1909) and Scott (1914). Scott (1914) described the regeneration within whole animals following self-evisceration, beginning with a stage 9 days after evisceration. Previously, Torelle (1909) described briefly the regeneration within posterior halves of bisected *Thyone* but described earlier stages than did Scott. We have then no account of the early stages of regeneration within whole animals and only a brief note on these stages as found in the regeneration within posterior halves. Furthermore, disagreement on the part of these two authors as to certain details in the later stages of regeneration raised the question as to whether regeneration within the whole animal is comparable to that within a posterior half. Thus the problem was twofold: (1) to ascertain the manner in which *Thyone* regenerates autotomized parts with especial reference to the early stages of such reconstitution and (2) to compare the regeneration occurring within a whole animal with that which takes place within a posterior half. With these two general problems in mind, a study was made of the regeneration within whole animals fol-

[1] This work was begun at Woods Hole in the summer of 1931 through the aid of a Collecting Net Scholarship.

lowing induced evisceration and also of the regeneration within anterior and posterior halves of *Thyone,* resulting from a cut made before or after evisceration.

I am deeply indebted to Dr. B. H. Willier for helpful advice and criticism given throughout the course of the investigation.

Auto-evisceration in Holothurians

There is much variation among holothurians in respect to the ease with which autotomy is induced. In *Stichopus regalis* the process is apparently a normal one taking place naturally every year toward the end of the summer (Bertolini, 1932*a*). In the laboratory this species will eviscerate readily when irritated by some means such as increased temperature of the water. It often exhibits autotomy when it is caught (Bertolini, 1930*b*). In speaking of *Holothuria captiva* Ludwig, now *H. parvula* Selenka (Deichmann, 1930, p. 70), Crozier (1914, p. 199) states that " even when kept in well ærated sea water aquaria protected from the light, holothurians will eviscerate after about four days."

In contrast to such forms, *Thyone briareus* has been kept at least a month in running water aquaria without exhibiting autotomy. Furthermore, among some 400 medium-sized specimens which were examined during the course of this investigation I found none that was in a natural state of regeneration. Since *Thyone* are greatly reduced in volume after evisceration, regenerating animals may have been eliminated through this selection for size. Only a very large specimen would escape it. The time of year may be another factor. In the instances of *Stichopus regalis,* Bertolini (1932*a*) found individuals in stages of regeneration during some seasons while at other seasons none was in this condition. She reported that of 50 animals collected in the spring none was in a state of regeneration, yet out of 119 collected during September and October, 110 were regenerating and 7 had recently eviscerated. It is possible then that regenerating *Thyone* might be collected in other seasons even though none was found in mid-summer. Pearse (1909) has stated that " it seems improbable that autotomy is an important factor in the daily life of the members of either genus " (i.e., *Thyone* or *Leptosynapta*).

Methods for Inducing Evisceration in Thyone

In order to obtain a complete series of animals showing progressive stages in the process of regeneration, it was necessary to find a stimulus effective enough to initiate autotomy but which would not lead to the death of the animal. Pearse (1909) injected various chemicals

into the body-cavity of *Thyone briareus*. He found that many substances which produce "intense contractions of the muscles of the body-wall, did not bring about ejection of the visceral organs" and "under the best of conditions it appears in only 35 per cent of the possible cases." The highest percentage of evisceration was obtained by the use of strychnine, seven out of twenty showing autotomy and living for at least a day. Scott (1914), working with this same species, induced autotomy by "allowing *Thyone* to stand in stagnant water until the water became foul." This was followed by "treatment with running water containing much oxygen. Alternating these processes produced as high as 65 per cent of self-mutilated individuals." These animals lived and regenerated the lost parts. It has been shown (Kille, 1931) that if *Thyone* were placed in dilute ammonia water autotomy almost invariably followed. If the animals were returned to sea water following this treatment, 96 per cent of them lived and regenerated the lost parts. In this same note (1931) it was also stated that electrical stimulation applied to the muscles of the body-wall will produce autotomy. Electrical stimulation proved to be as satisfactory as the use of ammonia water except that the ammonia method was the more rapid. Recently an electrical stimulus has been used successfully to induce evisceration in several species of Holothuria (Bertolini, 1932*b*) which, like *Thyone,* seldom if ever undergo autotomy.

Evisceration Induced by Ammonia Water

When *Thyone* is disturbed the anterior region of the body-wall is invaginated by the contraction of the five retractor muscles extending from the radial plates of the lantern to the longitudinal muscles of the body-wall (Figs. 1 and 2). The introvert (the invaginated anterior

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EXPLANATION OF PLATE I

Photographs 2, 8, 9, and 10 from preserved material; all others from motion picture film of living *Thyone* by J. R. Brewster. All figures 4/5 natural size except Figs. 2 and 7, which are 3/5.

FIG. 1. *Thyone briareus* with introvert retracted. Anterior end to the right. *p,* position of the posterior border of the introvert.

FIG. 2. Dissection to show position of introvert. *a,* anterior border of the introvert.

FIGS. 3, 4, 5, AND 6. Successive stages (intervals of 2 seconds) in the eversion and distention of the introvert during autotomy.

FIG. 7. Bursting of the introvert (at *b*). Small portion of the intestine visible.

FIG. 8. Autotomized anterior end of *Thyone* and viscera.

FIG. 9. Autotomized viscera partially disentangled in order to show lantern (*l*), polian vesicle (*p. v.*), stomach (*s*), tentacles (*t*), and intestine (*i*).

FIG. 10. An eviscerated *Thyone* opened along the right dorsal interambulacrum. Water lungs have been removed. Arrows indicate the mesentery. *g,* gonad tubules; *c,* cloaca.

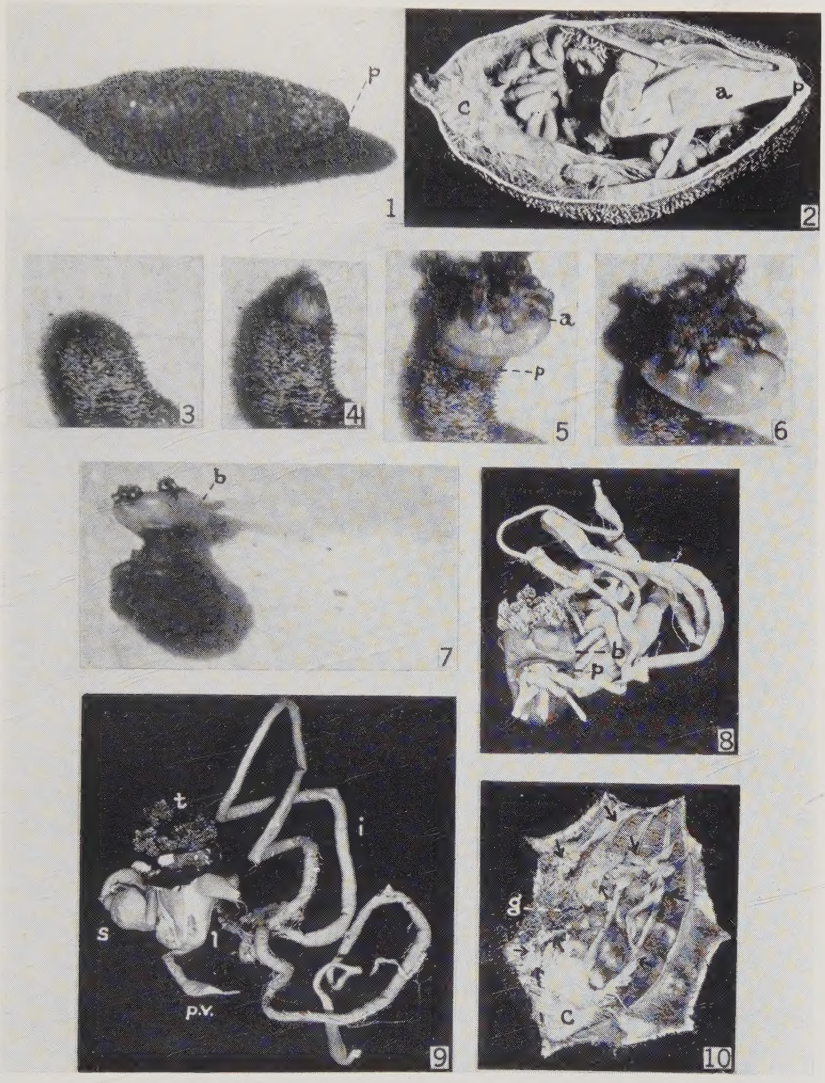

PLATE I

region of the body-wall, Fig. 2, *a-p*) possesses no tube feet and lacks the strong circular muscles which characterize the rest of the body-wall. The animal in this condition was submerged in weak ammonia water. Ammonium hydroxide was used in the proportion of one part 7 N. ammonia to 800 parts of sea water. Within about 15 seconds all body muscles are thrown into strong contraction as a result of which the body-wall is much wrinkled. The animal becomes reduced to a minimum size and assumes a spherical shape. In this condition the antero-posterior axis is about one-third the length of that of a fully extended specimen.

This period of extreme contraction continues for some 15 seconds. At the close of this period the invaginated anterior end becomes rapidly everted (Figs. 3, 4, and 5) and then greatly distended (Fig. 6) as the intestine, stomach, lantern, and body fluid are forced anteriorly into this region by the further contraction of the strong circular and longitudinal muscles of the body-wall. It is impossible to say at just what moment the retractor muscles lose their attachment to the longitudinal muscles of the body-wall and the gut is separated from its mesentery and cloaca. In order to throw some light upon these points three specimens were killed in the act of autotomy by the injection of 20 cc. Bouin's fluid into the body-cavity. In all specimens the introvert was evaginated and partly distended when fixed. Dissection showed that in one specimen all retractor muscles were detached but the posterior section of the intestine was not. It still retained its connection to the cloaca and its mesentery. On the other hand, in another specimen one retractor (the ventral) was still attached but the entire gut free from mesentery and cloaca was lodged in the expanded introvert. The third specimen, unlike the first, had only two detached retractor muscles but resembled the first in that the posterior end of the gut was still joined to the cloaca and its mesentery. It would seem from this evidence that there is no absolute sequence between the autotomy of the retractors and the autotomy of the gut, both events being completed some time during the last few seconds of the period of intense muscular contraction preceding evagination of the introvert or during the early stages in the autotomy of the body-wall following rupture. Furthermore, it is apparent that the actual separation of all the retractors from the body-wall does not necessarily precede the evagination of the introvert.

The distended introvert usually ruptures (Fig. 7, *b*). The body-fluid gushes forth carrying with it variable amounts of the viscera. The circular muscles of the body-wall contract so that the body-cavity is closed posterior to the rupture (region *p*, Fig. 2). The ruptured anterior end containing the viscera is sloughed off within several hours,

but can be very easily pulled away from the rest of the body immediately after its distention. When artificially completed in this way the whole process of autotomy usually takes place within a period of 35 seconds.

The place of rupture varies. If it occurs in the anterior (Fig. 5, *a*) or middle region of the introvert (Fig. 7, *b*) then the rupture of the body-wall has a position independent of its autotomy. Autotomy of the body-wall always occurs at the transition zone between the thin-walled introvert and the muscular body-wall (Fig. 5, *p*). Figure 8 shows the autotomized parts from a specimen in which the rupture of the body-wall (at *b*) and the region of autotomy (at *p*) are independent of each other. However, the distended introvert may burst either in the region of autotomy (*p*) or so close to it that autotomy of the body-wall is completed by the extension of the original rupture.

Evisceration Induced by Means of Electrical Stimulus

This method satisfactorily induced autotomy but was more time-consuming. The animals had to be stimulated individually whereas in the case of ammonia water a group could be treated at one time. Furthermore, the electrical stimulus had to be given for a somewhat longer period of time before autotomy occurred. The procedure consisted in applying a tetanizing faradic current to various parts of the body-wall. The application was made by means of two platinum wire electrodes which were about 2 mm. apart. The anterior, posterior, and middle regions of the body were touched successively but without any regard to a specific order. In the mid-body region the applications were made on or near all five radii which mark the position of the longitudinal muscles. As soon as the muscles contracted in the region stimulated the electrodes were moved to a new position, thus keeping all muscles of the body in a state of partial or complete contraction. Following a period of intense contraction self-evisceration occurred as has already been described for the ammonia method.

The Results of Autotomy in Thyone Briareus

The autotomized parts are much entangled and are partially covered by the introvert (Fig. 8). When these parts are disengaged (Fig. 9) one sees that they include the entire length of the intestine[2] with its hæmal vessels; the stomach; the introvert; the lantern with its associated structures. Under this latter category are included water-vascular

[2] For the present purposes the terminology of Coe (1912) and others will be retained. The so-called intestine thus includes at least three regions which are morphologically distinct in the living animal.

ring; Polian vesicle or vesicles; stone canal; madreporite; tentacles; œsophagus with its investment of calcareous plates; nerve ring; and the retractor muscles of the lantern. I have never seen the gonads ejected but this has been reported for an unidentified species of *Thyone* (Noll, 1881).

Examination of the autotomized parts also shows that they are usually eviscerated in the order of their body position and not according to their anatomical sequence. Lantern and stomach are eviscerated first, followed by the much-coiled intestine of the two regions *B* and *F–H* (Fig. 11) which are interconnected by hæmal vessels, then intestine in region *C–E,* and last of all that from the region *H–J*.

After autotomy of the viscera the animal consists of the body-wall minus the introvert; a cloaca with the attached respiratory trees; the two gonadal tufts, one on either side of the dorsal mesentery; and nearly

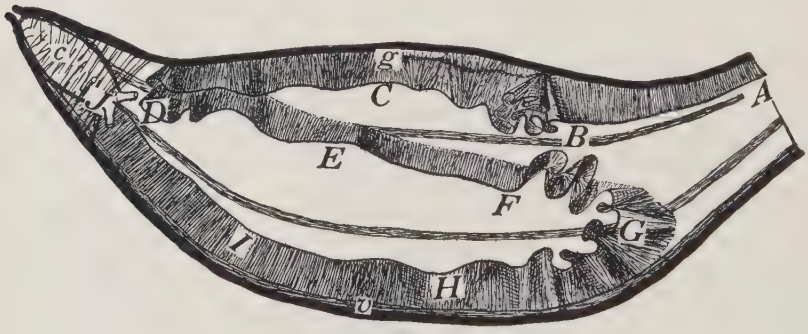

Fig. 11.

all of the mesentery. This condition following self-evisceration is seen in Fig. 10. The specimen has been cut open longitudinally along the right dorsal interambulacrum. The respiratory trees have been removed at the point of their attachment to the cloaca in order that the distribution of the mesentery might be more easily followed. The situation is more clearly seen in Fig. 11. The respiratory trees and the entire left side of the body-wall have been removed from an eviscerated specimen. For convenience in description ten arbitrary points have been designated along the free edge of the mesentery by the letters *A* through *J*. For purposes of orientation three other structural features are marked: namely, the region of the gonad attachment (*g*), the cloaca (*c*), and the ventral longitudinal body muscle (*v*). The diagram represents the animal in its typical shape. Actually, however, the animal assumes a more spherical shape following evisceration. There is also

a decrease in volume due to loss of much of the cœlomic fluid. As a result the mesenteries hang in abundant folds and the straight-line distance from the anterior end to the cloaca is reduced one-half or more.

Since the regeneration of the intestine involves the free edge of the mesentery, it is advisable to follow its distribution in detail. For the purpose of description the mesentery in *Thyone* may be divided into three sections. The first (dorsal) section of the mesentery attached to the dorsal interambulacrum extends from the anterior end of the body nearly to the cloaca (Fig. 11, *A–D*). The anterior third of this section (region *A–B*, Fig. 11) supported the lantern and the short, muscular stomach. The remainder (*B–D*, Fig. 11) suspended the anterior part of the intestine. These original relationships can be established when examining an eviscerated *Thyone* by referring to the hæmal vessel in the dorsal mesentery which extends from the gonads to the hæmal vessels of the alimentary canal. In the process of evisceration this strand of hæmal tissue is severed near its attachment to the viscera. The severed end at the free edge of the mesentery (posterior to *B*, Fig. 11) marks approximately the original position of the junction between stomach and intestine. The second (lateral) section of the mesentery which supported the middle third of the gut turns to the left, passes ventrally and anteriorly across the two left longitudinal muscle bands to a point far anterior (Fig. 11, *D–G*). At this point (Fig. 11, *G*) the mesentery makes a turn to the right and as the third section (ventral) runs posteriorly to the cloaca in the left ventral interambulacrum along the ventral longitudinal muscle (Fig. 11, *G–J*). This section supported approximately the posterior third of the intestine. An occasional specimen is found in which the third section of the mesentery is attached in the right ventral interambulacrum instead of the left. Such a distribution was described by Coe (1912).

Preparation of Materials

Using the two methods described above, 150 *Thyone* were eviscerated and immediately placed in running sea water. Five failed to survive the treatment. Of those which lived, groups of six were killed and fixed in Bouin's or Zenker's fluid at time intervals of 1 day up to an 11-day regeneration, and then at every second day to a regeneration of 32 days. In the process of fixation two methods were used. In the one first adopted the animal was cut open longitudinally through the right dorsal interambulacrum so as not to disturb the mesenteric attachments to the body-wall. The specimen was sewed or pinned out flat upon a piece of cork so that the entire body cavity was exposed.

The whole preparation was then turned upside down upon the fixing solution. In the older stages of regeneration a few cuts were made through the intestine to aid in fixation. By this method the regenerated viscera were fixed entire and in those specimens which had regenerated for 12 days or more all relations were preserved.

For the earlier stages of regeneration this method could not be followed because it fragmented and distorted the fragile rudiments and frequently pulled the mesenteries from their attachments. The procedure adopted for such specimens was as follows. A small amount (4–6 cc.) of Bouin's fluid was injected directly into the body-cavity. The needle of the syringe was inserted through the right body-wall so as to avoid the mesenteries. The animal was then immersed in Bouin's fluid. The tendency for the animal to contract was offset by the slightly increased cœlemic pressure due to injection. After a short interval a small window was cut through the body-wall on the right side. This was carefully enlarged until through it the mesenteries and primordia of the new alimentary canal could be seen. When their position was determined nearly all the body-wall could be removed from the right side without danger of their mutilation. The animal was then placed in fresh Bouin's. This method not only preserved the parts in their proper relations but also in their typical position. It proved so satisfactory that it was eventually adopted for fixation of all stages of regeneration.

In the main, the data as compiled in the present paper were obtained by means of an examination of the fixed specimens under a binocular dissecting microscope. Histological analysis of the regenerating tissues is not offered at this time but certain preliminary findings of this nature will be included.

Regeneration Within an Eviscerated Thyone

Early Stages in the Regeneration of the Stomach-intestine (2 to 10 days)

With the aid of a dissecting microscope ($\times$ 23) one can see a fine thread of opaque tissue along the edge of the mesentery in specimens which have regenerated only 2 days. This opaque thread appears distinct with a diameter slightly greater than the thickness of the mesentery. The margin of the mesentery is quite even or regular as compared with the more or less ragged, irregular free edge resulting from the autotomy of the gut. Four and 5 days after evisceration the early morphological indication of the regenerating alimentary tract can be seen with the naked eye. When examined under the dissecting microscope it appears in the form of a continuous rod-like thickening of the

free edge of the mesentery from *A* to *J*. The diameter of the rod is fairly constant but with some local variations here and there (Fig. 12, *I*). Evidently the entire free edge of the mesentery early exhibits a physiological activity which within 4 days finds morphological expression in the form of this rod-like thickening of the mesenteric edge.

While the edge of the mesentery early exhibits stages of regeneration, it also undergoes a general reduction in its total length. It will be recalled that the antero-posterior axis of the animal following evisceration is greatly reduced. The mesentery is thrown into many folds which are particularly noticeable in regions *D, G,* and just posterior to *B* (Fig. 11) where the mesentery formerly supported small loops of the intestine. These folds of the mesentery are gathered into numerous small tucks at the free margin where they are hemmed by the fine, straight thread or rod of regenerating tissue. In this way the length of the free edge of the mesentery becomes considerably reduced. The tucks are present in regenerations of 4 and 5 days but become much more prominent as regeneration advances.

Correlated with this general reduction in marginal distance from *B* to *J* there is a noticeable increase in the distance from the free edge of the mesentery to the line of attachment at the body-wall. While this growth in width is somewhat general it is pronounced in regions throughout *C–D–E* and *F–G–H*. One can easily see by referring to Fig. 11 that if growth occurs in these regions, and if at the same time the free edge is gathered into numerous tucks, the more or less hairpin turns of the free edge at *D* and *G* would be replaced by a gentle *S*-shaped curvature (Fig. 13, *B–D′–G′–I*). This *S*-shaped curve of regenerating mesenteric margin occupies a body level formerly occupied by only the middle third of each section of the mesentery. In this way the marginal distance from *B* to *J* has been further reduced. Some specimens which had regenerated for a period of 6 and 7 days showed this condition well advanced (Fig. 12, II) and it was characteristic of all specimens examined 8 and 9 days after evisceration (Fig. 12, III). In some instances (Fig. 12, IV) the rudiment of the stomach-intestine is almost a straight rod connecting the healed anterior end and the cloaca.

For detailed analysis of the marginal primordium eight specimens were selected, two for each regeneration period of 4, 6, 8 and 10 days. After examination under the binocular microscope the rudiments were removed and studied in greater detail by means of serial sections. Figure 12 shows four of these rudiments drawn to scale as seen from the right side. The mesenteries have been omitted but the ten letters

assigned to arbitrary points of the free edge of the mesentery in Fig. 11 are placed along the mesenteric side of the rudiments to indicate the approximate position of these same points. No detail of the lantern is attempted. It will be seen that the rod-like primordium of the alimentary canal 4 days after autotomy has a diameter varying between the extremes of .05 mm. and .23 mm. and averaging about .11 mm.

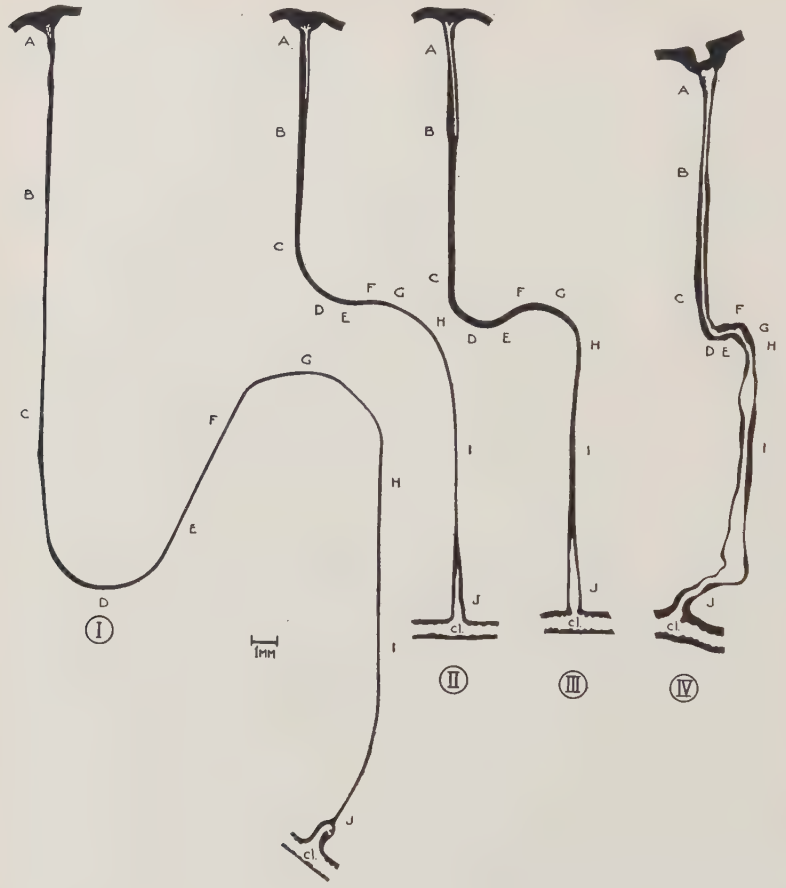

Fig. 12.

(Fig. 12, *I*). The anterior section of the rudiment is larger than that adjacent to the cloaca. There is no morphological indication here that this thickening at the edge of the mesentery was progressively established as an anterior growth from the cloaca as has been suggested for *Thyone* (Torelle, 1909). On the contrary, it would indicate a nearly equivalent activity throughout the length of the mesentery granting

slight advantage to the anterior third. When the rudiment from specimen *A* was sectioned it was found to be a solid rod of connective tissue covered by a much-thickened mesothelium. At the posterior end it was continuous with the wall of the cloaca at a point where the original intestine was torn away. Here the lumen of the cloaca ended blindly. At the healed anterior end, however, certain masses of cells appear to be penetrating this solid rudiment in a posterior direction. They extend as a number of finger-like projections from irregular masses of cells located around and between the healed anterior extremities of the ambulacral structures. The large size of the nuclei and the granular cytoplasm of these invading cells stand in sharp contrast to the surrounding connective tissue cells. They seem to be more intimately associated with the water vascular canals than any other structures. Within these cords of cells which thus penetrate the extreme anterior end of the stomach-intestine anlage to a depth of .7 mm., small intercellular spaces occur.

Serial sections of Specimen II reveal clearly that the primitive lumen for the new alimentary canal arises in two centers. One center is located at the extreme anterior end within the lantern rudiment and extends posteriorly. The forerunners of this lumen are the small intercellular spaces noted in Specimen I. This section of lumen measures .8 mm. at its greatest diameter and extends longitudinally for 2.7 mm. Cross sections show it to be roughly circular in outline throughout most of its extent with a simple epithelium composed of cells identical in appearance with those composing the solid cord-like masses described above for Specimen I. Anteriorly the lumen occupies a central position within the rudiment but as it passes posteriorly it moves toward the anti-mesenteric margin so as to take an eccentric position. The second center for the origin of a lumen is found at the extreme posterior end of the rudiment extending anteriorly from the cloaca for a distance of 2 mm. and is lined with an epithelium which is continuous with that of the cloaca. Approximately 17 per cent of the total rudiment possesses a lumen in this specimen.

In Specimen III the invasion of the solid rudiment is more advanced. The position and size of the two sections of lumen are indicated in the figure. Twenty-eight per cent of the total length now contains a lumen. In another specimen which had regenerated for 8 days this value was 45 per cent.

In Specimen IV there is one continuous lumen from the anterior end to the cloaca. A plate of connective tissue still closes off the lumen of the canal at the extreme anterior end. This does not persist much longer, for as early as 16 days after evisceration a mass of dark gray

ingested material was observed in both the stomach and intestine of two specimens. All specimens which had regenerated 20 days gave this macroscopic evidence of ingestion. One individual of this period was seen feeding in the aquarium.

Later Stages in the Regeneration of the Stomach-intestine (12 to 32 days)

About 12 to 14 days after evisceration the regenerating stomach-intestine shows an enlargement for a short distance posterior to the lantern anlagen. This is the forerunner of the muscular stomach. At 10 days the transition from the stomach region to the intestine is a gradual one. In one specimen at 12 days, and in all specimens of a longer period of regeneration, there is an abrupt transition from an anterior enlargement to the smaller diameter characteristic of the intestine. In these instances the stomach anlage is sharply marked off from the intestine. Eighteen days after evisceration, the stomach in most specimens is not only a distinct region of the regenerating tract but it also shows externally an extremely muscular posterior section typical of the normal.

Macroscopically the later changes in the intestine are mainly those resulting from increased length due to growth in certain regions. These intestinal regions are comparable to those in the normal animal where the intestine is thrown into numerous small loops. This is noted to some extent just posterior to *B* (Fig. 11) and also in the region of *G*. It is especially marked in the region of *D*. Just as in the normal, the regenerated intestine in these regions is early thrown into small loops so that the regularity of the *S*-curved gut is lost. It will be recalled that in the early stages of regeneration the free edge of the mesentery is gathered into numerous small tucks in these three regions, greatly reducing the length of the mesentery edge. Now, as the regenerating intestine at the margin of the mesentery grows in length, these tucks or folds become smoothed out and tend to disappear. The increased length of the intestine is first noticeable in the region *D′* (Fig. 13) which is approximately at the gonad level in a specimen of 14 days regeneration. As this loop of the intestine lengthens it extends farther and farther posterior from the gonad and is eventually thrown into a number of minor loops. In one 18-day regeneration the intestine extended posterior from the gonad and showed two distinct, small loops (*D″*, Fig. 13).

Concurrently with this growth in length of the intestine from *D′* to *D″* the intestinal hæmal plexus is established within the first major loop.

At 14 days one can identify within the first large elbow of the *S*-curved gut, a crescent-shaped membrane which in texture resembles the mesentery (Fig. 13, *X–Y–Z*). One slim horn of the crescent (*X–Y*) extends far anterior along the anti-mesenteric surface of the first main section of the intestine while the other tapering horn is attached to the anti-mesenteric surface of the second major section of the gut (*Y–Z*). The posterior convex margin of the crescent is attached to the anti-mesenteric surface of the gut loop (*D'*). The concave anterior margin of the crescent is free (*Y*). Both the distribution and the nature of the membrane soon change. In two specimens regenerated for 18 days the curved gut in the region *D''* (Fig. 13) is no longer attached to the posterior, convex margin of the crescent. The gut in this region has

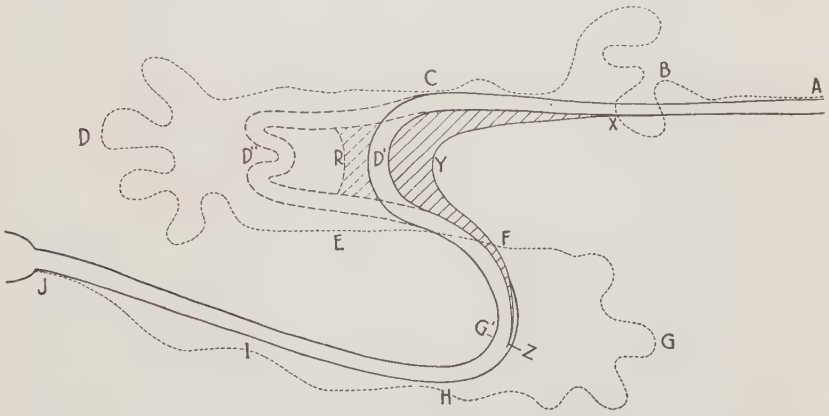

FIG. 13. *A–D–G–J*, the free edge of the mesentery as in Fig. 11; *A–D'–G'–J*, the relative position of a regenerated intestine 14 days after autotomy; *A–D''–G'–J*, th same 20 days after autotomy.

extended its length posteriorly from the gonad and apparently the crescent membrane has not kept pace. Both the anterior and the posterior borders of the membrane are now free (*Y* and *R*). Furthermore, the tissues of the membrane show condensations in the form of a plexus of strands. In the two specimens being discussed much of the membrane intervening between these strands has disappeared, leaving a net-like plexus. As a result of these two changes the former crescent-shaped membrane now extends across the body-cavity as a bridge-like plexus connecting the first two major sections of the intestine. This is the forerunner of the intestinal hæmal plexus.

Stages in regeneration from 22 to 32 days show no marked changes except the continued elongation of the intestine and a general increase

in size of all rudiments. None of the specimens had a regeneration period to exceed 32 days. Scott (1914, p. 293) states that "Thyone A, killed at 41 days, was practically a normal, both in behavior and appearance, except for the fact that the regenerated organs had not yet reached full size."

Regeneration of the Lantern and Associated Structures

Immediately after autotomy of the viscera, the circular muscles of the body-wall in the region of *p* (Fig. 2) contract strongly so as to close the body-cavity anteriorly. Within 24 hours most specimens have successfully healed the wound by means of a small circular, plate-like connective tissue mass.

The events occurring at the healed anterior end are best seen when the animal has been dissected so as to gain an internal, posterior view of the tissues in that region. From this aspect one can see the broken ends of all five longitudinal muscles which peripherally border upon the central connective tissue platelet. The anterior extremities of these muscles mark the points at which the ambulacral structures were torn across at the time of autotomy of the body-wall. The central platelet is probably derived from the neighboring connective tissue of the body-wall. The free edge of the dorsal mesentery terminates anteriorly in a fusion with the center of this tissue mass. From these three sources, the anlagen of the lantern structures arise.

As early as 5 days after autotomy one notes that each of the five radial water-vascular canals has budded a pair of small lateral vesicles at the level where the longitudinal muscle terminates, and in addition has extended its own length beyond that termination. As each canal grows anteriorly, all five canals converge centripetally towards a central point on the posterior surface of the connective tissue platelet while the paired lateral vesicles enlarge, establishing a ring of ten vesicles around its margin. These ten vesicles are the rudiments of the tentacles, which therefore arise as buds from the radial canals.

As the growing tips of the radial canals converge toward a common center, each branches dichotomously. The branches unite to form a "pentagonal canal" as has been described and figured by Scott (1914, p. 292, fig. 4).

> "The anterior ends of the radial canals fork dichotomously, and these branches anastomose to form the canal which later assumes a circular shape around the esophagus."

Usually by the time such anastomoses occur the free edge of the mesentery continuous with the center of the connective tissue platelet has

developed a definite thickening so that the canal surrounds a definite enlargement of the mesentery margin from which will develop a part of the œsophagus. The appearance of the lantern anlagen at this stage reminds one of a dished-in wagon wheel on the end of an axle—the axle in this case being the solid rod-like thickening of the free edge of the mesentery, the hub being prospective ring canal, the spokes represented by the five radial canals of the lantern, while the ten tentacular vesicles indicate the circumference of the rim. When the radial canals first branch, their forked ends lie in nearly the same plane as do the tentacular vesicles, a plane which would be at right angles to the antero-posterior axis of the animal. The lantern rudiment in these early stages is therefore quite flattened, with the tentacular vesicles and ring canal forming two concentric circles about the free edge of the mesentery at *A* (Fig. 11). As regeneration advances, it is quite clear that the pentagonal canal surrounding the regenerating alimentary tract at the mesentery edge is moving more and more posterior from the plane passing through the tentacular vesicles. By the time the pentagonal canal has assumed the circular shape characteristic of the water-vascular ring, the lantern rudiment is no longer flattened but has acquired a definite antero-posterior length. The proportion is still not that of the normal lantern. In the typical lantern the maximum diameter is roughly one-half the length, while the ratio of these measurements of a regenerating lantern possessing a well-formed ring canal and tentacular vesicles may be exactly the reverse. The apparent movement of the ring canal posteriorly is associated seemingly with a relative greater growth in the region of the lantern between the ring canal and the tentacular vesicles, a region crossed by the five radial canals. At all events, the lantern rudiment eventually changes from a somewhat flattened tissue mass in which structures lie nearly in a plane at right angles to the body axis, to the form of an inverted truncated cone with its base attached anteriorly to the body-wall and its body proper projecting posteriorly into the body-cavity.

The tentacular vesicles mark the anterior level of the lantern anlagen, while the ring canal lies at the posterior limit just as in the normal adult lantern. As stated above, the tentacular vesicles arise near the termination of the longitudinal muscles. It results then that the five longitudinal muscles of the body-wall are attached peripherally at five points about the anterior end of the regenerating lantern. As early as 8 days after evisceration one can see that the longitudinal muscles have started to split off a slender inner strand from the main muscle mass. These strands of muscle become the retractor muscles of the lantern as described by Scott (1914). The splitting apparently begins

a short distance from the lantern, and continues towards the lantern and posteriorly as regeneration advances. The anterior limit is soon reached at the termination of the longitudinal muscle. The splitting continues posteriorly along each longitudinal muscle thereby constantly increasing the length of the retractor muscles. All four specimens which have regenerated 14 days possessed retractor muscles about 4 mm. long. They were present in all later stages. In no specimen was there ever observed any signs of retractor muscle regeneration in the regions where the original retractors severed their attachments to the longitudinal muscles.

Twelve to 14 days after evisceration ten centers of calcification appeared anteriorly in the lantern establishing a minute calcareous ring. The ten centers mark the positions of the five radial and the five interradial plates of the completely formed lantern.

Polian vesicles originate as interradial evaginations of the water vascular ring. They can be identified in all specimens which have regenerated 12 days or more. The number of vesicles varies from one to three, as is true of the adult. The stone canal apparently originates as a dorsal diverticulum of the ring canal and is seen projecting into the dorsal mesentery. In a specimen which had regenerated 14 days the lengths of two Polian vesicles are .2 mm. and 1.2 mm. and the diameters are .12 mm. and .64 mm. respectively. The stone canal has a diameter of .12 mm. and a length of 1 mm. The distal blind end of the stone canal is slightly enlarged. In a specimen which had regenerated 16 days, that part of the dorsal mesentery between the stone canal and the regenerating lantern has disappeared so that the stone canal now borders the edge of the dorsal mesentery which hangs free in the mid-lantern region. Specimens at 28 days have a well developed calcareous madreporite plate of typical form.

By 14 days then, almost all rudiments of the conspicuous lantern structures are established. Most of these appear as a miniature of the typical structure. There are of course certain differences in shape so that even if the small size were ignored, the lantern at this stage could still be identified as being in the process of regeneration. The first specimen was seen feeding by means of its small, unpigmented, regenerated tentacles 20 days after autotomy.

Regeneration Within Posterior Portions of Thyone

The earliest paper on regeneration in *Thyone briareus* was that of Torelle (1909). This investigator cut off and discarded the anterior part of the body. She found that the posterior part which contained

" the intestine, the reproductive organs, and the respiratory trees " could regenerate new anterior structures. Obviously such an operation removes a bit more of the body-wall than the amount lost through the process of autotomy. Furthermore, the resulting posterior part might possess a portion of the intestine although Torelle found that " in many individuals the intestine was autotomously severed near the cloaca immediately after the operation." In spite of the reduction in the amount of body-wall and the presence of a portion of the original intestine, one would expect that such posterior halves would regenerate in much the same manner as does an eviscerated, whole animal. Yet in this brief note quite a different picture is given. Because of these differences it was thought desirable to again study regeneration within posterior sections of *Thyone briareus*.

Two series of animals were prepared. In Series *A*, the anterior ends of twenty-four eviscerated animals were amputated by a transverse cut at the level of the gonad (Fig. 11, *g*). Since there is no external indication of the attachment of the gonad, there was some variation in the cut. In all cases the lantern and associated structures were removed and sometimes the gonads. One remnant of the mesentery left within the posterior portion has a *C*-shaped distribution (Fig. 11, *C–D–E*) while the other remnant is the posterior portion of the ventral mesentery (*H–I–J*). The same operation was performed upon twenty animals of Series *B* but these were uneviscerated *Thyone*. The major difference between the two groups was that in series *B* the posterior halves retained more or less of the original intestine. This series was a repetition of Torelle's procedure. Immediately following the operation the two parts of the animal were placed in a finger bowl of running sea water. The anterior parts of the animals composing Series *B* died within 96 hours after the operation but nine of those from Series *A* lived until they were killed for examination. In the case of four anterior halves this was a period of 36 days. The posterior halves from each series possessed an equivalent viability. Approximately 80 per cent survived the operation in each instance.

Examination of the posterior portions of eviscerated *Thyone* (Series *A*) revealed a type of regeneration entirely in agreement with that observed within a whole animal. The complete rod-like rudiment of the stomach-intestine is established by an early activity which always involves the entire free edge of the mesenteric remnants. If the cut was made considerably posterior to the level of the gonad, then the rudiment along the remnant of the ventral mesentery (*H–I–J*) was easily found but that formed in the region of *D* was inconspicuous as a small portion at the extreme anterior end of the animal, i.e., immedi-

ately continuous with the regenerating lantern. This latter contribution is not readily recognized in the early stages of regeneration unless the animal is opened with extreme care. The slightest strain on the section of the mesentery *C–D–E* will pull it away from the rudiment which is held in place by its attachment to the cloaca, to the healed anterior end, and to the remainder of the mesentery. As a result the rudiment of the alimentary canal appears to be a contribution entirely from the remnant of the ventral mesentery, *H–I–J*. Later stages are less fragile. These show clearly that all mesentery posterior to the level of the cut plays a part in the establishment of the alimentary canal. This is more easily seen when the cut is at the level of the gonad attachment or just anterior to this point. The portions of the dorsal and left lateral mesenteries which were posterior to the cut form a rod-like thickening at their free edge. When viewed from the right side this thickening resembles a *C*. The rudiment along the edge of the remnant of the ventral mesentery appears as a straight rod, the anterior end of which is fused to the ventral arm of the *C*. Thus the typical *S*-shaped distribution of the original intestine is regained although imperfectly at first. Instead of a letter *S*, the rudiment resembles a reversed numeral 2.

These regenerating posterior portions further resemble regenerating whole animals in respect to the origin of the intestinal lumen. As was the case in whole animals, this lumen was found within the otherwise solid thickening of the mesenteric margin in two widely separated regions. One section is adjacent to and continuous with the cloaca, while the other is within the anterior portion of the rudiment, extending posterior from the lantern primordium. Thus the primitive lumen of the intestine is not established as a progressive development from the cloaca although the posterior portion may so arise. The lantern regeneration is usually delayed some 10 days as compared with its reconstitution within a whole animal. It was commonly observed that these animals transversely bisected in the region of the gonad experienced some difficulty in healing shut the cut end. This delay may account for the longer period required by a posterior half to regenerate a lantern. The retractor muscles are formed as in a regenerating whole animal.

Observations on the twenty specimens composing Series *B* agree with those on Series *A* when allowance is made for the parts of the original intestine which may remain. In four specimens such remainders consisted of only a short length attached to the cloaca. In the others a variable amount of the original gut was retained but never all of that which originally occupied the posterior half of the animal. Wherever a piece of the original intestine remained attached to the

mesentery, this piece was incorporated into the rudiment of the new alimentary tract. Thus sections of old gut may alternate with newly regenerated links.[3] The mesenteric edge wherever free eventually bore a rod-like thickening of regenerating tissue. In some instances, sections of the original intestine became incompletely detached from the mesentery so that one end was still held by the mesentery while the other end projected into the body-cavity. That part which remained attached to the mesentery was incorporated as a link in the newly formed intestine. Usually the part extending into the body-cavity ended blindly. The new digestive system may in this way receive contributions from uneviscerated parts of the old intestine, although the origin of a lumen within the newly regenerated sections is not dependent upon such parts.

As noted above, nine anterior halves from Series *B* lived until they were killed for examination. In one of these at 11 days after the operation a small rod-like rudiment was identified along the edge of the mesentery. Such evidence from these isolated anterior halves shows that the formation of the rudiment is entirely independent of the cloaca or any posterior remnant of the original alimentary canal. The anterior halves which had regenerated for 36 days showed a well developed lantern and associated structures, stomach, and intestine but there was no indication of a cloaca.

Discussion

In respect to the regeneration of the lantern proper, my observations support those of Torrelle (1909) and Scott (1914), namely, that it regenerates from the severed anterior ends of the five sets of ambulacral structures. However, these investigators give two very different accounts of the manner in which the lantern retractor muscles arise. When examining regeneration within posterior portions of *Thyone,* Torelle (1909, p. 20) observed that:

> "if short pieces of the old lantern muscles have been left attached to the longitudinal muscles at the time of operation, the ends of these begin to proliferate new tissue about the time that the new lantern forms. . . . If the animal had been divided just posterior to the lantern-muscles, so that these had been entirely removed, a proliferation of the tissues of the longitudinal muscles takes place, in the form of a bud, at a point homologous with the position of the lantern-muscles in the normal animal. In either case the new muscles grow forward in three or more separate strand-like parts, which unite into one just before union with the lantern is effected."

[3] This has also been observed in three whole animals in which small remnants of the original intestine had been retained.

In contrast to this account, Scott (1914, p. 291) observed that within a whole *Thyone* following autotomy " the anterior end of each of the longitudinal muscles had split off a very slender branch to form a new retractor muscle (see fig. 3)." On this point my findings in the cases of both posterior halves and whole animals support those of Scott.

In regard to the formation of a new intestine, I find that regenerative activity begins along the entire free edge of the original mesentery. A rod-like thickening of the mesenteric margin is well developed 4 days after autotomy, forming a continuous rudiment extending from the healed anterior end to the cloaca. This analysis is quite different from that presented by Torelle (1909). Studying regeneration within posterior halves, she observed that " the new intestine always forms as a bud from one side of the old intestine, at a point near the cloaca " and it " grows forward as a solid rod of cells from one to two millimeters in diameter " until it " has become attached to the anterior closed end of the body-wall." Then " it elongates and turns on itself forming the loops characteristic of the normal animal." In contrast to this emphasis upon the importance of remnants of the original alimentary tract in the establishment of the primary rudiment, I would stress the potentialities of the mesentery throughout its full extent in whole animals as well as in posterior halves. There is evidence that a contribution is made by the cloaca in the formation of the primitive epithelium of the posterior portion of the intestine but none to indicate that this solid rod of cells at the margin of the mesentery forms as an anterior growth from a cloacal bud. If it does, it must complete its anterior progression within 2 days after autotomy. Such rudiments would be microscopic while the " solid rod of cells " described by Torelle was " one to two millimeters in diameter." The fact that a solid rudiment forms at the edge of the mesentery in an anterior half of *Thyone* which was cut off immediately following autotomy, demonstrates that neither cloaca nor any other remnant of the original alimentary canal need be present for its origin.

The question of the origin of the intestinal epithelium is, of course, another matter. It remains to be seen whether or not the intestinal epithelium which arises in an anterior half is equivalent to that within a regenerating whole *Thyone*. The evidence indicates that in whole *Thyone* an intestinal epithelium and lumen is progressively established within the posterior levels of the solid rudiment by an anterior invasion of cells from the cloacal epithelium. We have seen, however, that this contributes but a portion of the intestinal epithelium, for anteriorly the primary, solid rudiment is invaded by cells which arise within the regenerating lantern. While the exact source of these cells has not as yet been determined, the possibility that they come from any remnant

of the original alimentary canal is excluded. Nothing but body-wall and mesentery is present in this region after autotomy.

The statement (Torelle, 1909, p. 19) "as soon as attachment to the anterior closed end of the body-wall is effected" the new intestine "elongates and turns on itself forming the loops characteristic of the normal animal" raises a puzzling question as to the changes that must take place in the mesentery. It is possible that this conclusion was reached as a result of observation of regeneration along the straight edge of the ventral mesentery (*H* to *J*, Fig. 11) and a failure to note the part played by the other mesenteric remnant (*C* to *E*). When the rôle of the mesentery is recognized, the eventual distribution of the new intestine is easily understood. Obviously, if the regeneration of the new intestine involves the entire edge of the mesentery which formerly supported the original intestine, then the new alimentary tract will "form the loops characteristic of the normal animal." In respect to the primary rudiment, most of the difficulties of interpretation are, therefore, eliminated when one thinks in terms of the potencies of the mesentery rather than contributions from a remnant of the original alimentary tract, i.e., the cloaca.

The part played by the mesentery in the establishment of a solid rudiment connecting the healed anterior end with the cloaca is a consistent one whether in whole animals or parts of animals. No situations have been observed where one could be absolutely sure that the rudiment ever departed from the mesentery to grow out through the cœlom independently. Every instance where this seemed to be a possibility proved otherwise. Upon careful examination it was seen that most of these misleading conditions were artificially produced as a result of too great a tension on the mesentery during fixation or examination. In early stages of regeneration the slightest tug upon the mesentery may pull it free from the rudiment at its margin. This is particularly true of the material while it is still alive. Scott (1914) noted that the new alimentery canal in a regeneration of 9 days is located along the mesenteric margin but Torelle (1909) does not mention the mesentery in her account of the earlier stages. Bertolini (1932*b*), in a passage cited below, has described for the genus *Holothuria* a situation in which the rudiment growing posteriorly from the anterior end leaves the mesenteric margin, cuts across the cœlomic cavity to another section of the mesentery, and continues on its way. It thus entirely avoids a certain region of the original mesentery but rejoins the mesentery at another point. To date I have observed no instance of this in *Thyone briareus*. In an attempt to test the possibility, sections of the mesentery were removed but in all these cases the mesentery grew rapidly from minute

fragments which remained close to the body-wall and a typical regeneration followed. In carrying out this particular operation, animals which had eviscerated were turned inside out so that the mesenteries hung free in the water. Sections of the mesentery which were removed were trimmed as close to the body-wall as possible with the aid of fine shears. The animals were then turned rightside out.

The formation of the lantern at the healed anterior end of the body is probably independent of any regenerative processes occurring in various levels of the mesentery posterior to it. Torelle (1909, p. 19) pointed out that during regeneration within posterior halves the lantern never began to form "before the intestine had become united to the body wall," i.e., to the body-wall of the anterior end. The comment is made that "the attachment of the intestine appears to be a stimulus which results in . . . a proliferation of cells" to form "the beginning of a new lantern." This statement loses all significance when one finds that there is no forward growing intestine and therefore no moment when it becomes "united to the body wall." The rudiment adjacent to the body-wall in this region is as well developed in all stages as that along the mesentery at any other level. The apparent dependence of the lantern upon the regenerated intestinal rudiment within a posterior half of *Thyone* can be explained entirely upon the basis of a time relationship existing between the closure of the anterior end of the body and the activity of regeneration along the edge of the ventral mesentery. As has been stated above, the posterior portion of a *Thyone* resulting from a bisection at the level of the gonad requires considerably more time to successfully heal the wound and close the body anteriorly than does a whole animal following autotomy. Regeneration of the lantern is delayed some 10 days. On the other hand, this transverse cut does not proportionally decrease the activity of the edge of the ventral mesentery. The result is that in most specimens there will be established a sizable primordium of the intestine from the level of the cut to the cloaca before healing and regenerative processes will have established the lantern anlagen.

The recent investigations of Bertolini make it possible to compare the regeneration of the new intestine in *Thyone briareus* with like processes in the genera *Stichopus* and *Holothuria*. When *Stichopus regalis* undergoes autotomy, the cloaca tears. The intestine along with the respiratory trees and reti mirabili are emitted through the anus. In the *Holothuria* the left respiratory tree is retained. In both genera the œsophagus and cloaca remain in the body. The digestive system of *Stichopus regalis* is not regenerated from these remnants of the original tract, however, but forms in the mesentery at the expense of cells

which are not distinguishable from other cells of the mesenchyme (Bertolini, 1930*b*). Specimens which had just undergone autotomy could not be kept alive in the laboratory for more than a day or so. Fortunately, Bertolini was able to secure from the sea four animals in various stages of regeneration. In the first stage an irregular cavity was found in the mesentery at its free margin. No internal epithelium lined this cavity and its aspect was practically the same at all levels. The second stage described is that of a small transparent tube seen macroscopically to run the full length of the mesentery. A definite internal epithelium was seen in a third animal which represents a still later stage in regeneration. The author points out that in all three specimens the development is equivalent at all levels of the mesentery. From this the deduction is made that the epithelium of the new intestine did not have its origin from that which remained in the œsophagus and cloaca.

In contrast to this, in *Holothuria tubulosa* (Bertolini, 1932*b*), a thin, transparent tube grows posterior from the œsophagus, avoids all the mesentery which formerly held the loop of the stomach [4] and passes along the length of the mesentery which previously held the intestine. At the same time another thin tube with a blind end starts from the cloaca and runs forward along the mesentery which formerly held the last portion of the original intestine. After about 60 days the two tubes meet and unite to form a single tube extending from the œsophagus to the cloaca. At 4 months the digestive apparatus appears to be normal again. Successive, late stages were not obtained so that the steps in the regeneration of the stomach are unknown. We do not know, therefore, whether the mesentery which finally supports the new stomach is the old mesentery or a new one.

In these two genera we see quite different methods in regeneration. *Thyone briareus* presents a third variation, for it has something in common with each, yet it presents a distinct mode of regeneration of its own. In respect to the establishment of the primary solid rudiment extending from the healed anterior end to the cloaca, *Thyone* somewhat resembles *Stichopus*. That is, the entire edge of the mesentery becomes active without much variation at different levels. This is not true for the origin of the intestinal lumen and epithelium. In respect to these, *Thyone* resembles the genus *Holothuria* in that they arise in two centers which are located at the extremities of the animal. The closer homology is found in the origin of the epithelium from the cloaca. In respect to the origin of the epithelium at the anterior end the similarity is one of position only, for in *Thyone* there is no anterior remnant of the original

[4] Nomenclature of Enriques (1902) was used.

alimentary tract to contribute the invading cells which establish the anterior section of the intestinal epithelium. At the present stage of investigation I am unable to state the source of these invading cells. In the region where they are first seen we have the healed severed ends of all the ambulacral structures which include muscle, nerve, water-vascular and the so-called hæmal tissue as well as the mesentery.

It is of interest to recall that the embryonic origin of the water-vascular system is a vesicle which at an early stage separated from the primitive archenteron. Both intestinal epithelium and the lining of the water-vascular system are therefore closely related ontogenetically. However, one should build no argument on this platform, for the fact that structures which embryonically arise from one germ layer may regenerate from a different one has long ceased to occasion surprise. From the numerous examples the situation in the nemerteans may be called to mind. Coe and others have reported that if an anterior piece of a nemertean be cut off at such a level that it contains none of the digestive system, this piece will reconstitute a complete worm. The new midgut is formed from the mesenchyme and phagocytic cells of a posterior blastema (Coe, 1934).

Summary

1. Autotomy of the stomach, intestine and lantern with its associated structures was uniformly induced in *Thyone briareus* by chemical or electrical stimulation. Ninety-six per cent of the animals lived and regenerated the lost parts.

2. Although the body-wall may burst in various regions of the introvert, actual autotomy of the body-wall always occurs at the junction between the introvert and the muscular body-wall.

3. The earliest macroscopic indication of the regenerating digestive system takes the form of a continuous, rod-like thickening of the free margin of the mesentery connecting the healed anterior end and the cloaca.

4. A lumen arises within the extremities of the primary, solid rudiment. The intestinal epithelium lining the posterior section of lumen is continuous with the cloacal epithelium while at the anterior end the intestinal epithelium arises from certain masses of cells identified within the lantern rudiment.

5. The solid rudiment is progressively invaded from these two centers until 10 days after autotomy the two sections of lumen have joined to form a continuous lumen for the new alimentary canal.

6. The lantern and associated structures originate from contributions

of the body-wall and more especially from the anterior extremities of the ambulacral structures.

7. A posterior half of an eviscerated or uneviscerated *Thyone* will regenerate all missing structures in a manner entirely comparable to that occurring within an entire animal except that regeneration of the lantern is delayed.

8. Remnants of the original intestine remaining in posterior halves cut from uneviscerated animals may become incorporated as links in the newly-formed digestive system. This has also been observed within incompletely eviscerated whole animals.

9. Anterior halves of eviscerated *Thyone* killed at 37 days after the operation possessed a well developed lantern with its associated structures, stomach, and intestine but no cloaca.

10. A comparison is made between the methods of regeneration found in the genera *Stichopus, Holothuria,* and *Thyone.*

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